



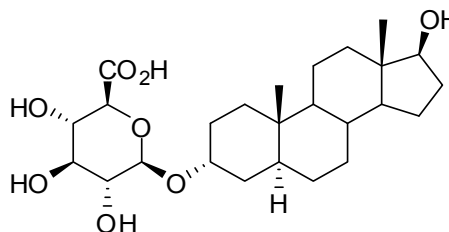
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S004: 5 α -Androstan-3 α ,17 β -diol-3-O- β -glucuronic acid

Report ID: S004.2017.02

Chemical Formula: C₂₅H₄₀O₈

Molecular Weight: 468.6 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
09-S-08	65535-18-4	83.5 \pm 2.8%

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit ($k = 2$).

IUPAC name: (3 α ,5 α ,17 β)-17-Hydroxyandrostane-3-yl β -D-glucopyranosiduronic acid

Expiration of certification: The property values are valid till 27 June 2022, i.e. five years from the date of re-certification provided the **unopened** material is handled and stored in accordance with the recommendations below. The material as issued in the unopened container and stored as recommended below should be suitable for use beyond this date, subject to confirmation of batch stability from the issuing body. The expiry date/shelf life does not apply to sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: White powder prepared by synthesis, certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

Recommended storage: When not in use this material should be stored at or below 4 $^{\circ}$ C in a closed container in a dry, dark area.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

Stability: This material has demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials.

This material has shown signs of long term instability in methanol solution. While not confirmed, we believe this to be the result of esterification to the methyl ester.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC with ELS detection on ten randomly selected 1-2 mg sub samples of the material. The material was judged to be sufficiently homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% confidence level from that observed on repeat analysis of the same sample.

Safety: Treat as a hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
11 February 2020

This report supersedes any issued prior to 11 February 2020

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

CIPM MRA notice: This certificate is consistent with the capabilities that are included in Appendix C of the CIPM MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognize the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in Appendix C. The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate (for details see <http://www.bipm.org>).

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by quantitative nuclear magnetic resonance (qNMR). The one-proton multiplet at 3.94 ppm was measured against a certified internal standard of maleic acid.

Supporting evidence is provided by elemental microanalysis, HPLC-ELSD and ¹H NMR.

QNMR:	Instrument:	Bruker Avance-500
	Field strength:	500 MHz
	Solvent:	MeOH- <i>d</i> ₄ (3.31 ppm)
	Internal standard:	Maleic acid (98.7% mass fraction)
	Initial analysis:	Mean (3.94 ppm) = 84.8%, s = 0.8% (5 sub samples, October 2011)
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 μ m (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (65:35) The aqueous phase was buffered at pH 2.3 using formic acid
	Flow rate:	1 mL/min
	Detector:	ELSD-LT II detector
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.4%, s = 0.2% (10 sub samples in duplicate, October 2011)
	Re-analysis:	Mean = 99.6%, s = 0.1% (5 sub samples in duplicate, October 2012)
	Re-analysis:	Mean = 99.7%, s = 0.1% (5 sub samples in duplicate, September 2013)
	Re-analysis:	Mean = 99.5%, s = 0.04% (5 sub samples in duplicate, September 2014)
	Re-analysis:	Mean = 99.6%, s = 0.06% (5 sub samples in duplicate, June, 2017)
Thermogravimetric analysis:	Non volatile residue < 0.2% mass fraction (June 2011). The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis.	
Karl Fischer analysis:	Moisture content 11.8% mass fraction (October 2011) Moisture content 13.1% mass fraction (October 2012) Moisture content 13.5% mass fraction (September 2013) Moisture content 13.2% mass fraction (September 2014) Moisture content 13.5% mass fraction (July 2017)	

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters 2695 (HPLC)/Micromass Quatro
	Column:	X-Bridge C-18, 100 mm \times 2.1 mm I.D. \times 3.5 μ m
	Column temp:	45 $^{\circ}$ C
	Solvent system:	200 mM NH ₄ ⁺ HCO ₂ ⁻ (aq) buffered to pH 3 [5% v/v], Methanol [52% v/v], MilliQ water [43% v/v]
	Flow rate:	0.2 mL/min
	Sample prep:	50 μ g/g in MeOH/MilliQ water (25:75)
	Injection volume:	30 μ L
	Ionisation mode:	Electrospray negative ion
	Capillary voltage:	3.5 kV Cone voltage: 45 V
	Source temp:	130 $^{\circ}$ C Desolvation gas temperature: 350 $^{\circ}$ C
	Cone gas flow rate:	23 L/hr Desolvation gas flow rate: 753 L/hr
	The retention time of 5 α -androstan-3 α , 17 β -diol-3-O- β -glucuronic acid is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	16.25 min:	467.2 (M-H ⁺) <i>m/z</i>
GC-MS:	The free steroid was liberated upon treatment with β -glucuronidase enzyme (E. Coli K12) and derivatised with MSTFA.	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 1, 17 m \times 0.22 mm I.D. \times 0.11 μ m
	Program:	180 $^{\circ}$ C, 3 $^{\circ}$ C/min to 240 $^{\circ}$ C, 10 $^{\circ}$ C/min to 265 $^{\circ}$ C, 30 $^{\circ}$ C/min to 310 $^{\circ}$ C
	Injector:	260 $^{\circ}$ C Transfer line temp: 300 $^{\circ}$ C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	14/1
	The retention time of the <i>bis</i> -TMS derivative of 5 α -androstan-3 α , 17 β -diol is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	<i>Bis</i> -TMS (10 min):	436 (M ⁺ , 13), 421 (10), 346 (22), 331 (24), 256 (32), 241 (55), 215 (31), 148 (23), 129 (73), 107 (28), 93 (26), 81 (27), 73 (100) <i>m/z</i>
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm ⁻¹ , KBr powder
	Key peaks:	3423, 2930, 1717, 1446, 1362, 1261, 1162, 1066, 1035 cm ⁻¹
¹ H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	MeOH- <i>d</i> ₄ (3.31 ppm)
	Spectral data:	δ 0.72 (3H, s), 0.78 (1H, m), 0.83 (3H, s), 0.87-1.05 (3H, m), 1.12-1.71 (15H, m), 1.78-1.88 (2H, m), 1.96 (1H, m), 3.24 (1H, dd, <i>J</i> = 7.9, 9.3 Hz), 3.38 (1H, t, <i>J</i> = 9.2 Hz), 3.53 (1H, dd, <i>J</i> = 9.8, 9.0 Hz), 3.56 (1H, t, <i>J</i> = 8.6 Hz), 3.76 (1H, d, <i>J</i> = 9.7 Hz), 3.94 (1H, m), 4.37 (1H, d, <i>J</i> = 7.8 Hz) ppm
	Formic acid and an unidentified steroid estimated at 0.2% and 0.4% mass fraction respectively are observed in the ¹ H QNMR.	
¹³ C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	101 MHz
	Solvent:	MeOH- <i>d</i> ₄ (49.0 ppm)
	Spectral data:	δ 11.7, 11.9, 21.5, 24.3, 26.5, 29.5, 30.6, 32.8, 33.7, 35.3, 36.9, 37.0, 38.1, 40.5, 44.1, 52.5, 55.8, 73.2, 74.8, 75.7, 76.6, 77.6, 82.6, 103.0, 172.6 ppm
Melting point:	N/A	
Microanalysis:	Found:	C = 56.6%; H = 8.4% (October, 2011)
	Calculated:	C = 64.1%; H = 8.6% (Calculated for C ₂₅ H ₄₀ O ₈)
	Calculated:	C = 56.5%; H = 8.9% (Calculated for C ₂₅ H ₄₀ O ₈ + 11.9% water)
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Methanol/chloroform (1/2) Single spot observed, R _f = 0.35. Visualisation with vanillin